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(54) Title: CANCER CHEMOTHERAPEUTIC CYCLIC AND ACYCLIC DISULFONIC ESTER COMPOUNDS, METHOD OF USE THEREFOR, AND INTERMEDIATES

(57) Abstract

Bifunctional cyclic disulfonic ester compounds effective in treating certain types of cancers. Initial nucleophilic reaction with the compound produces a negatively charged sulfonic acid end-group which remains attached to the compound until a second alkylation reaction can occur.

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CANCER CHEMOTHERAPEUTIC CYCLIC AND ACYCLIC
DISULFONIC ESTER COMPOUNDS, METHOD OF USE
THEREFOR, AND INTERMEDIATES.

1. **Field of the Invention**

The present invention relates to bifunctional
alkylating compounds; in particular, to cyclic
disulfonic ester alkylating compounds.

2. **Background**

Alkylating agents are a major class of cancer
chemotherapeutic compounds. Most clinically used
alkylating agents are bifunctional compounds having two
chemically reactive centers capable of reacting with and
either fragmenting or cross-linking biomolecules, such
as the opposite strands of duplex DNA, or DNA and
associated protein. Use of these agents to alkylate
biomolecules leads to a variety of defects in
intracellular metabolism, particularly defects in
nucleic acid replication and/or transcription, which
tend to be more lethal in rapidly growing cancer cells
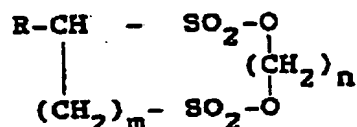than in normal, somatic cells.

One class of bifunctional alkylating agents
include linear, uncharged disulfonates, exemplified by
Busulfan (Myleran, Burroughs Wellcome), which is
commonly used in the treatment of leukemias. Guide to
Therapeutic Oncology, Bergevin, P. R., et al, eds,
Williams and Wilkins, Baltimore/London (1979), p 110. A
compound of this type acts through an initial
nucleophilic reaction with a target biomolecule, such as
a DNA base, alkylating the target and splitting off a
free, charged sulfonic acid group. The uncharged
sulfonic-ester alkylating group may then participate in
a second nucleophilic reaction, to crosslink the
original target molecule to a second target and split

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off a second sulfonic acid group. That is, th
bifunctional reaction scheme involves an uncharged
intermediate alkylating group. The 1,4-butanediol
sulfonate (Bulsulfan) is more effective therapeutically
5 than shorter or longer alkyl-chain linear sulfonates.

3. Summary of the Invention

The present invention includes cyclic
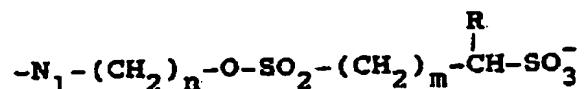disulfonic esters having the general structural formula:

$$R-CH - SO_2-O$$
$$(CH_2)_n$$
$$(CH_2)_m- SO_2-O$$

10 where $m=0$ or 1, $n=1-5$, and $R=H$, CH_3, CH_3CH_2 or
Cl. The compounds of the invention are useful as
bifunctional agents for fragmenting biomolecules, such
as DNA, or cross-linking a variety of nucleophilic-
containing biomolecules, such as nucleic acids and
15 associated proteins. The cyclic disulfonic ester in
which $m=0$, $n=2$ and $R=H$ is effective in the treatment of
a variety of animal cancers, including mammalian
lymphocytic leukemia, lymphoid leukemia,
melanocarcinoma, human breast xenograft and ovarian
20 carcinoma. Cyclic disulfonic esters in which $m=0$, $n=3$
or 4 and $R=H$ have also been shown to have anti-leukemic
activity.

Unlike linear disulfonic esters (where the
butanediol compound is most therapeutically effective)
25 among cyclic compounds of the present invention, the
disulfonic esters of ethanediol ($n=2$) and pentanediol
($n=5$) appear to be the most active in treating leukemic
animals. The cyclic esters of the present invention are
also shown herein to be effective in treating a variety
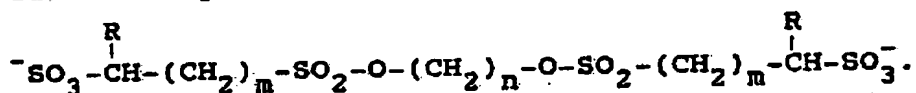30 of cancer types other than leukemias, particularly

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m lanocarcinomas, breast xenografts and ovarian
carcinomas.

Also unlike uncharged linear alkane
disulfonates such as Busulfan, initial nucleophilic
attack on a cyclic diester compound of the invention, in
opening the diester ring, results in an intermediate
linear sulfonate having a charged sulfonic acid
end-group. In this regard, the invention more generally
includes a bifunctional cross-linking agent capable of
reacting with a first nucleophile-N_1 to produce a
nucleophile-reactive intermediate of the form

$$-N_1-(CH_2)_n-O-SO_2-(CH_2)_m-\overset{\overset{\displaystyle R}{|}}{C}H-SO_3^-$$

where n, m and R are as identified above. The agent may
be a cyclic compound of the type described above or a
linear compound of the form

$$^-SO_3-\overset{\overset{\displaystyle R}{|}}{C}H-(CH_2)_m-SO_2-O-(CH_2)_n-O-SO_2-(CH_2)_m-\overset{\overset{\displaystyle R}{|}}{C}H-SO_3^-.$$

The invention further includes methods for
synthesizing cyclic disulfonic ester compounds of the
type described. In one method, useful particularly for
synthesizing the n=1 cyclic disulfonic ester of the
above structure, an alkanedisulfonyl chloride is allowed
to react with a silver salt to form the corresponding
silver disulfonate, which is then allowed to react with
a dihaloalkane, such as dibromoethane or diiodomethane.
In a second method, useful in the preparation of n=2-5
compounds, an alkanedisulfonyl chloride is allowed to
react directly with an alkanediol in the presence of an
aliphatic or aromatic tertiary amine, such as
triethylamine or collidine. The tertiary amine is added
dropwise to the other reactants at a low temperature to
avoid alkylation of the amine by the product ester.

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Another aspect of the invention includes a
method of treating mammalian cancers, particularly
lymphocytic and lymphoid leukemia, melanocarcinoma,
human breast xenograft and ovarian carcinoma, which
5 comprises treating the cancerous mammal with a
therapeutically effective amount of the cyclic
disulfonic acid compound.

A general object of the invention is to provide
a new class of cross-linking compounds which are
10 therapeutically effective in the treatment of several
types of cancer.

Another object of the invention is to provide a
disulfonic ester bifunctional cross-linking agent
capable of reacting with a first nucleophile to produce
15 a nucleophile-reactive intermediate having a charged
sulfonic acid end group.

Yet another object of the invention is to
provide a class of cyclic bifunctional alkylating
compounds which are readily synthesized in yields
20 suitable for drug testing and manufacture.

These and other objects and features of the
invention will become more fully apparent from the
following detailed description of the invention and
accompanying examples.

25   Detailed Description of the Invention
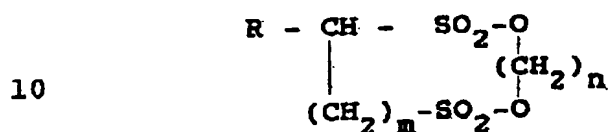The cyclic disulfonic esters of the present
invention are synthesized according to novel methods
described below in Section I. Section II describes the
reaction of cyclic disulfonic esters with DNA, to
30 produce DNA strand breaks and DNA cross-linking to
associated proteins. The section also considers a
linear disulfonic ester having charged sulfonic acid end
groups. This compound may react with duplex DNA in a

manner similar to that of the cyclic compound. A method
of synthesis of the linar charged cross-linking agent
is also described. Various drug treatment regimens in
which selected cyclic disulfonic ester compounds are
5 employed in the treatment of five different types of
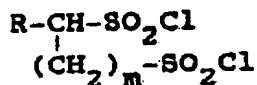mammalian cancers are outlined in Section III.

I. **Synthesis of Disulfonic Ester Cross-linking Agents**
The present invention includes cyclic
disulfonic esters having the general structural formula:

$$R - CH - SO_2-O$$
$$| \qquad\qquad (CH_2)_n$$
$$(CH_2)_m-SO_2-O$$

where $m=0$ or 1, $n=1-5$, and $R=H$, CH_3, CH_3CH_2, or
Cl.

The first synthetic method to be considered is
particularly suitable for the synthesis of compounds of
15 this type in which $n=1$. The method generally includes
allowing an alkane disulfonyl chloride of the form:

$$R-CH-SO_2Cl$$
$$| $$
$$(CH_2)_m-SO_2Cl$$

where m and R are as indicated above, to react with a
silver salt, preferably silver carbonate, under
20 conditions which produce the corresponding silver
disulfonate. Experimentally, the reaction is preferably
carried out in the dark under completely anhydrous
conditions. An alkane disulfonylchloride, such as
methanedisulfonyl chloride, is dissolved in a suitable
25 solvent, such as acetonitrile, and to this solution is
added a silver salt, such as silver carbonate, in a
molar ratio of slightly more than two moles of silver
per mole of the disulfonyl chloride. The mixture is
kept below 40°C during the initial exothermic reaction
30 and is then stirred at room temperature for 24 hours.

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The silver chloride powder which forms is removed by
filtration. The reaction method, which is described in
Example I below, yields approximately 88.5% of the
theoretical yield of silver methanedisulfonate.

5   In the second step of the synthesis, the
freshly prepared silver alkanedisulfonate is allowed to
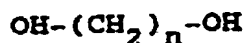react with a dihalide of the form:

X-(CH$_2$)$_n$-X

where n=1-4 and X is either bromide or iodide. By way
10   of illustration, silver methanedisulfonate dissolved in
a suitable solvent, such as acetonitrile, is added to
the dihalide, in approximately a 1 to 1 molar ratio, and
the mixture is allowed to stand for up to several weeks
at room temperature or is heated under reflux for up to
15   several days in the absence of light. The precipitated
silver salt is filtered and the filtrate is evaporated
under reduced pressure, leaving a typically light brown,
oil-like residue containing the desired product. The
residue is dissolved in a suitable solvent, such as
20   methylene chloride, and may be treated with a purifying
agents such as decolorizing charcoal added to the
solvent. To crystallize the product, a second solvent,
such as cyclohexane, is added until a cloudy supernatant
forms. Recrystallization in a solvent system such as
25   cyclohexane:methylene chloride 2:1 may be carried out to
achieve a desired purity. The identity of the product
can be confirmed by characteristic infrared (IR)
features, such as CH$_2$ and SO$_2$ stretching
frequencies, and by proton nuclear magnetic resonance
30   (NMR) features, such as the resonance positions of the
CH$_2$-SO$_2$ proton, the end-CH$_2$-O proton and the
middle CH$_2$ proton. Comparing experimental product
elemental analysis with theoretical values provides
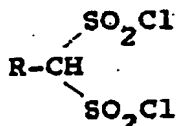further product-identity confirmation.

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Example II describes the prepartion of
tetram thylene methan disulfonate (m=0, n=4, R=H), also
named 1,5,2,4-dioxadithiocane-2,2,4,4-tetroxide, from
1-4 dibromobutane and silver methanedisulfonate. The
5   procedure yielded, upon recrystallization, small white
needles whose final weight represented an approximately
3.79% total yield. In Example III, the preparation of
trimethylene methanedisulfonate (m=0, n=3, R=HO, also
named 1,5,2,4-dioxadithiocane-2,2,4,4-tetroxide, from
10 silver methanedisulfonate and 1,3-dibromopropane is
detailed. An approximate 11% yield of small white
crystals identified as trimethylene methanedisulfonate
was obtained. Example IV describes the synthesis of
ethylene methanedisulfonate. Alternative procedures
15 described in this example gave yields, upon
recrystallization in a cyclohexane-methylene chloride
mixture, of 2.18% and 2.78%. The method of synthesis of
methylene methanedisulfonate (m=0, n=1, R=H), also named
1,5,2,4-dioxadithiane-2,2,4,4-tetroxide, is described in
20 Example V, and includes allowing silver
methanedisulfonate to react in acetonitrile with an
approximately equal molar amount of diiodomethane. A
total product yield of about 2.22% was obtained.

A second general method for the synthesis of
25 the novel cyclic disulfonate esters is particularly
suitable for compounds whose structures correspond to
those in which m=0 and n=2-5, and R=H or CH_3. The
method generally includes adding a diol of the form:

$$OH-(CH_2)_n-OH$$

30 where n=2-5, to a solvent such as tetrahydrofuran or the
dimethyl ether of ethylene glycol (glyme), and adding to
this solution, in the same solvent, an approximately
equal molar amount of an alkanedisulfonyl chloride of
the form:

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$$R-CH \begin{array}{c} \nearrow SO_2Cl \\ \searrow SO_2Cl \end{array}$$

where R=H or CH$_3$. The mixture is cooled to at least
about -20°C and an aliphatic or aromatic tertiary amine
is added dropwise to the mixture. Preferred tertiary
5   amines include triethylamine and collidine, a tertiary
aromatic amine. The reaction mixture is allowed to warm
to 0°C or slightly higher and the hydrochloride salt
which forms is removed by filtration. The filtrate is
evaporated under reduced pressure, and the residue,
10   which generally includes a light yellow oil, is
dissolved in a suitable solvent, such as methylene
chloride. A light crystalline powder, representing the
desired recrystallized product, is obtained by
crystallization in a suitable solvent system such as
15   methylene chloride:cyclohexane. The identity of the
product may be confirmed by characteristic IR and NMR
features, such as those mentioned above, and by
elemental analysis.

         Example VI below describes the synthesis of
20   pentamethylene methanedisulfonate (m=0, n=5, R=H), also
named 1,5,2,4-dioxadithiocane-,2,4,4-tetroxide,
according to the just-described method. A solution of
1-5-pentanediol in glyme was mixed with
methanedisulfonyl chloride in the same solvent, and to
25   this mixture was added triethylamine dropwise under
anhydrous conditions. After removing the amine
hydrochloride residue and evaporating the solvent, the
oily residue was redissolved in methylene chloride,
washed with 3 different aqueous wash solutions, and
30   crystallized from a methylene chloride:cyclohexane
solvent system. The procedure gave an approximately
6.75% yield of pure product. Examples VII and VIII

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describe similar reaction procedures for the synthesis
of ethyl ne methanedisulfonate (m=0, n=2, R=H) from
ethylene glycol and methanedisulfonyl chloride in
tetrahydrofuran, with the dropwise addition of

5   collidine. A 25% yield of recrystallized product was
obtained. Examples IX and X describe similar reaction
methods for producing trimethylene and tetramethylene
methanedisulfonate, respectively.
        Examples XI-XIV describe the synthesis of

10 1,1-ethanedisulfonates (m=0, R=CH$_3$) in which n=5
(Example XI), n=4 (Example XII), n=3 (Example XIII) and
n=2 (Example IX). It is noted that the cyclic
disulfonate compound in which n=1 cannot be synthesized
by the present synthetic method. In Example XI, a 2%

15 product yield of pentamethylene 1,1-ethanedisulfonate,
was formed. In Example XII, a 0.2% product yield of
purified tetramethylene 1,1-ethanedisulfonate, was
obtained. The method of Example XIII gave an
approximately 36% product yield of the trimethylene

20 1,1-ethanedisulfonate, and Example XIV produced a 25%
product yield of purified ethylene 1,1-ethanedisulfonate.
        It will be appreciated from the examples that
the general synthetic methods described can be modified
readily, particularly with respect to the

25 alkanedisulfonyl chloride starting material, to produce
compounds having various indicated R groups and m values.

II. The Cyclic Disulfonate Ester Alkylation Reaction
        The cyclic disulfonic ester compound of the
present invention has a chemically reactive center at

30 each of the CH$_2$-O group carbons, which is capable of
reacting with a nucleophile-containing biomolecule. The
initial alkylation reaction between a first nucleophile,
designated -N$_1$, and the cyclic disulfonic ester

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results in the formation of a linear intermediat of th
form:

$$-N_1-(CH_2)_n-O-SO_2-(CH_2)_m-\overset{\overset{\displaystyle R}{|}}{CH}-SO_3^-$$

having a negatively charged SO_3^- end-group. It can
5   be appreciated that the linearized alkylating
    intermediate has markedly different solubility and
    charge characteristics from those of the cyclic
    compound. It is expected that these charge and
    solubility characteristics will affect the configuration
10 which the compound adopts with respect to the alkylated
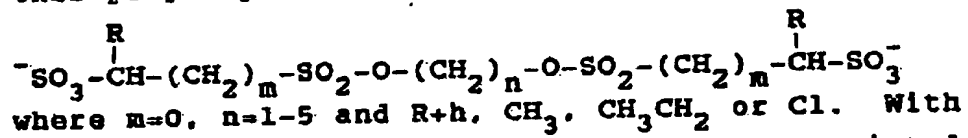   biomolecule. In particular, the charged end group may
    interact with positively charged histones associated
    with duplex DNA. Preliminary experiments conducted in
    support of the present invention indicate that the
15 cyclic ethylene disulfonic ester (n=2) is active in
    cross-linking DNA with DNA-associated proteins, in both
    human embryonic lung fibroblasts, cell line IMR-90, and
    its SV-40-transformed counterpart, cell line VA-13
    (which has lost the ability to repair small alkyl
20 lesions at the 06-position of guanine). Interestingly,
    little or no DNA/DNA crosslinking was observed in cells
    treated with the n=2 compound, although high levels of
    both frank strand breaks and alkali-labile lesions (pH
    12.6) were observed in both cell types treated with the
25 n=2 compound. The high levels of detected strand breaks
    and the lower toxicity seen in the IMR-90 cell line,
    which is capable of removing small alkyl lesions at the
    06 position of guanine, suggest that the strand breaks
    observed may not be drug induced, but induced by the
30 enzymatic repair activity.
           Following the initial alkylation event, the
    linearized charged complex can participate in a second
    nucleophilic reaction, with a second nucleophile N_2.

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forming a cross-linked-N_1-$(CH_2)_n$-N_2 complex, and releasing a second charg d sulfonic acid.

The present invention more generally contemplates disulfonic esters which are characterized

5 by a sulfonic acid end-group after an initial alkylation reaction. A class of linear disulfonic esters having this property have the general structural formula:

$$^-SO_3-\overset{\overset{\textstyle R}{\textstyle |}}{C}H-(CH_2)_m-SO_2-O-(CH_2)_n-O-SO_2-(CH_2)_m-\overset{\overset{\textstyle R}{\textstyle |}}{C}H-SO_3^-$$

where m=0, n=1-5 and R+h, CH_3, CH_3CH_2 or Cl. With

10 reference to this structure, it can be appreciated that initial nucleophilic attack by a nucleophile N_1 at an $O-CH_2$ carbon. produces an N_1-alkylating agent complex which is identical to the complex formed after the initial nucleophilic reaction involving the

15 corresponding cyclic disulfonic ester.

Example XV below outlines a procedure for the synthesis of 1,2-bis(oxysulfonylmethanesulfonic acid)ethane (m=0, R=H, n=2). In the method, methanedisulfonyl chloride is allowed to react with

20 water in the presence of diethyl ether to produce the corresponding chlorosulfonylmethanesulfonic acid. The sulfonic acid group in this chemical intermediate is protected by reaction with trimethylsilyl chloride or t-butyldimethylsilyl chloride, according to known

25 procedures. The compound is then allowed to react with ethylene glycol in a suitable solvent, such as glyme, with the addition of an aromatic or aliphatic tertiary amine such as triethylamine dropwise at -20°C. The product is treated with H_2O and bicarbonate salt to

30 hydrolyze the silyl esters in the compound, forming the desired salt of the product.

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III. Anti-cancer Activity of Cyclic Methanedisulfonate
      Esters

        The effectiveness of cyclic methanedisulfonate
esters against various types of mammalian cancers was
5 studied. Individual strains of mice identified as
having one of the following types of cancer were
employed in the study: lymphocytic leukemia, lymphoid
leukemia, melanocarcinoma, human breast xenograft and
ovarian carcinoma. For each type of cancer, a group of
10 animals all of about the same size and weight were
treated with one of a number of increasing dose levels
of the test drug, to identify optimal dosage levels, as
evidenced by either maximum survival period or
inhibition of tumor growth.
15   In each test, animals were divided into two
equal-number groups: a control group which received the
drug carrier alone; and the treated group which received
the drug, at optimal dose levels in the drug carrier.
In the studies involving lymphocytic leukemia, lymphoid
20 leukemia, melanocarcinoma, and ovarian carcinoma, drug
effectiveness was measured by the ratio of median number
of days the treated animal survived (T) to the median
number of days the control animal survived (C),
designated the T/C ratio. Drug effectiveness against
25 human breast xenograft was measured by the ratio of
tumor size for treated (T) to tumor size for control (C)
animals.
        The treatment protocol and results obtained for
ethylene methanesulfonate drug treatment are described
30 in Example XVI. The data show that ethylene
methanesulfonate substantially prolongs survival time or
inhibits tumor growth for all types of cancers which are
reported in the example.

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Two additional groups of mice having
lymphocytic leukemia were treated with either
trimethylene methanedisulfonate (n=3) of tetramethylene
methanedisulfonate (n=4) for purposes of comparing the
5 therapeutic effectiveness of cyclic disulfonic ester
compounds having n=2-4 cross-linking chain lengths. The
test conditions and protocol are substantially like
those employed in the Example XVI tests, and are
described in Examples XVII and XVIII, respectively, for
10 the n=3 and n=4 compounds. Both the trimethylene and
tetramethylene methanedisulfonate compounds showed
significant anti-leukemic activity, as measured by their
T/C ratios, but both were substantially less effective
than the cyclic ethylene methanedisulfonate ester in
15 prolonging survival in leukemic animals.

From the foregoing, it can be appreciated how
various objects of the invention are met. The cyclic
disulfonic esters described herein provide a new class
of cross-linking agents whose structure and reaction are
20 quite different from uncharged, linear disulfonic
esters. One of the novel compounds of the invention has
been shown to be useful in the treatment of a variety of
cancers including leukemias, ovarian carcinoma,
melanocarcinoma and breast xenograft.

25 The compounds of the invention are readily
synthesized by one or both of the methods detailed
herein, and for several of the compounds, product yields
greater than about 25% may be achieved.

The following examples illustrate various
30 methods of synthesis and treatment protocols associated
but are not intended to limit the scope of the invention.

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Example I
Preparation of Anhydrous Silver Methanedisulfonate

Completely anhydrous and dark conditions were
employed throughout the preparation. All glassware was
5 baked in an oven at 110°C for at least one-half hour.
Methanedisulfonyl chloride was synthesized according to
known methods; see. e.g., Schroeter, G., Ann Chem (1919)
161-257. Redistilled methanedisulfonyl chloride (2.00
g, 0.009 mole), was transferred in 15 ml of acetonitrile
10 obtained from Burdick and Jackson Laboratories
(Muskegon, MI) to an equalizing dropping funnel. The
acetonitrile was dried by distillation over P_2O_5.
Analytical grade silver carbonate obtained from J.T.
Baker Chemical Co. (Phillipsburg, NJ) (99.8%) was
15 weighed (5.22 g, 0.019 mole) and placed into a
three-neck flask fitted with an equalizing funnel, a
reflux condenser with a drying tube, and a thermometer.
A stir bar was added and the disulfonyl chloride
solution was allowed to drop in slowly. The mixture
20 became warm and a gas was evolved. The temperature was
kept below 40°C in an ice-water bath. The stir bar was
started as soon as possible and the mixture was stirred
at room temperature for approximately 24 hours. The
reaction mixture was filtered, yielding a light purple
25 powder containing silver chloride and unreacted silver
carbonate. The weight of the dried powder was 2.99 g,
0.29 g over the theoretical weight of silver chloride
assuming complete reaction of the silver carbonate.
Based on these numbers, the yield of silver
30 methanedisulfonate in the filtrate was calculated to be
about 88.5%.

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Example II

Preparation of Tetramethylene Methanedisulfonate

Purified 1,4-dibromobutane obtained from
Aldrich Chemical Co. (Milwaukee, WI) (2.03 g, 0.009
5   mole) was added to a flask containing 100 ml of freshly
prepared solution of silver methanedisulfonate in
acetonitrile from Example I. The flask was stoppered
and placed in the dark at room temperature for a period
of 8 weeks, during which a yellow-green precipitate
10 formed and settled to the bottom of the flask. The
suspension was filtered, and the filtrate was washed
with dry acetonitrile, leaving silver bromide in
suspension. The suspension was filtered and the
filtrate dried as in Example I. The dried weight of the
15 filtrate was 2.34 grams, or 67.7% of the theoretical
expected weight of silver bromide, based on complete
reaction of dibromobutane with the silver
methanedisulfonate.

The original filtrate was evaporated under
20 reduced pressure, leaving a light brown oil. Washing
the oil with methylene chloride turned the oil into a
brown gum and a cloudy supernatant. The supernatant was
decanted and treated with decolorizing charcoal.
Removal of the charcoal by filtration left a colorless,
25 clear solution. The solvent was removed under reduced
pressure, yielding small white cubic crystals. The
crystals were recrystallized from a 2:1
cyclohexane-methylene chloride mixture. Small white
needles were recovered, dried, and weighted. The weight
30 was 0.082 g, representing a 3.79% yield. The product
had a melting point at 143-144°C. NMR and IR spectral
analysis of the sample showed the spectral
characteristic expected for tetramethyl
methanedisulfonate. Elemental analysis of the product

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calculated for $C_5H_{10}O_6S_2$ is: C, 26.08; H, 4.38;
S, 27.85. The experimental values were C, 26.08, H,
4.77; and S, 27.66.

Example III
Preparation of Trimethylene Methanedisulfonate

5   Purified 1,3-dibromopropane obtained from
Aldrich Chemical Co. (4.76 g. 0.024 mole) was added to a
freshly prepared silver methanedisulfonate solution in
100 ml of dry acetonitrile from Example I. The mixture

10 was heated under reflux at 82° for 3 days, after which a
yellow-green powder formed. The powder was filtered,
washed with dry acetonitrile, filtered, dried and
weighted. The dry weight of 5.92 grams represented
65.5% of the expected weight of silver bromide, based on

15 complete reaction of the dibromopropane with silver
methanedisulfonate. The solvent from the reflux
reaction was removed under reduced pressure, and the
remaining oil was treated by the procedure described for
the purification of tetramethylene methanedisulfonate in

20 Example II. The small white crystals obtained weighed
0.563 grams, representing an 11% yield, and showed
melting points between 156 and 157.5°C and 185.5 and
186.5°C. NMR and IR spectra of the twice recrystallized
compound showed the characteristic features of

25 trimethylene methane disulfonate. The calculated
elemental analysis for $C_4H_8O_6S_2$ is C, 22.22; H,
3.72; and S, 29.66. The experimental values were: C,
22.31; H, 3.69; and S, 28.91.

Example IV
Preparation of Ethylene Methanedisulfonate

30   Purified 1,2-dibromoethane obtained from
Aldrich Chemical Co. (4.42 g. 0.024 mole) was added to a

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freshly prepared silver methanedisulfonate solution in
approximately 100 ml of acetonitrile, prepared in
accordance with Example I. After 4 days of heating
under reflux at 82°, the reaction mixture was cooled and
5 filtered. The yellow-green powder thus obtained was
washed with acetonitrile, dried, and weighed. The 4.01
gram weight of the dried powder represented 44.5% of the
expected weight of silver bromide, based on complete
reaction. The filtrate from the reflux reaction was
10 removed under reduced pressure, leaving a light brown
viscous oil. The oil was treated with methylene
chloride, as in Example II, forming a cloudy white
supernatant and an opaque brown gum. The supernatant
was decanted and treated with decolorizing charcoal and
15 diatomaceous earth. The solution, after filtering, was
clear and colorless. The solvent was removed under
reduced pressure leaving small white crystals. These
were recrystallized from a cyclohexane-methylene
chloride mixture and vacuum dried. The dried weight was
20 0.113 g, representing a 2.18% theoretical yield, and the
melting point was about 170°C. IR and NMR spectra of
the recrystallized product showed the characteristic
features of ethylene methanedisulfonate. Atomic
analysis was in conformity with the values calculated
25 for $C_3H_6O_6S_2$.

The same procedure described above was
repeated, using 5.09 g, 0.028 mole of purified
1,2-dibromoethane and a 100 ml solution of silver
methanedisulfonate. The reaction was heated under
30 reflux at 82°C for one day. The yellow-green powder
weighed 3.55 g, 0.75 g less than the weight of the
expected AgBr. Small white needles having a total
weight of 0.162 g, representing a 2.78% yield, were
obtained.

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Example V

Preparation of Methylene Methanedisulfonate

A flask containing approximately 100 ml of
silver methanedisulfonate solution was equipped with a
5 relux condenser and a drying tube. Purified
diiodomethane obtained from Aldrich Chemical Co. (5.09
g. 0019 mole) was added and the solution was heated
under reflux for 2 days. A light yellow powder which
formed was filtered, washed, and dried, as in Example
10 II. The dried precipitate weighed 5.79 g. representing
72.0% of the weight of the expected AgI. The filtrate
was treated as described in Example II to obtain small
white needles having a total weight of 0.081 grams,
representing a yield of 2.22%, and having a melting
15 point of between 146°C and 146.5°C. IR and NMR spectral
analysis of the white needles, after further
recrystallization, showed the features characteristic of
methylene methane disulfonate. Elemental analysis
calculated for $C_2H_4O_6S_2$ was: C, 12.76; H, 2.14;
20 and S, 34.09 for $C_2H_4O_6S_2$. The measured values
were: C, 12.91; H, 2.14; and S, 34.16.

Example VI

Preparation of Pentamethylene Methanedisulfonate

The present example and following Examples
25 VII-XII detail the synthesis of cyclic alkane disulfonic
esters by the reaction of an alkane disulfonyl chloride
with diols of the type: $HO-(CH_2)_n-OH$ where n=2, 3,
4 or 5.

Dimethyl ether of ethylene glycol (glyme)
30 obtained from Burdick and Jackson Laboratories was
purified by distillation over sodium and benzophenone.
A solution of 1,5-pentanediol obtained from Aldrich
Chemical Co. (12.5 g. 0.12 mole) in 350 ml of purified

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glyme was stirred in a 3-neck 1 liter round bottom flask
equipped with a stirrer and a thermometer. The reaction
flask was maintained at a temperature of -20°C by a
dowanol-dry ice bath. Methanedisulfonyl chloride,

5 prepared according to the method described by Fild, M.
and Rieck, H. P., _Chem Zeitung_ (1976) _109(9)_:391, (25.6
g, 0.12 mole), dissolved in 25 ml of glyme, was slowly
added through a 60 ml dropping funnel. A solution of
triethylamine obtained from Eastman Organic Chemicals

10 (Rochester, NY) (24.3 g, 0.24 mole) in 125 ml of glyme
was added dropwise to the vessel over a one hour
period. Care was taken to avoid contact with water by
covering the dropping funnels with drying tubes filled
with $CaCl_2$. Once all additions were complete, the

15 reaction mixture was allowed to return to room
temperature and stirred for two hours.

The reaction mixture was vacuum filtered to
remove the solid triethylamine hydrochloride. The solid
amine hydrochloride residue, which was washed with glyme

20 and then allowed to dry, weighed 37.0 g, representing a
104% calculated theoretical yield. The filtrate was
roto-evaporated below 37°C to remove the glyme. The
residue was redissolved in 100 ml of methylene chloride
and washed with the following series of cold, aqueous

25 washes: a) three times with 30 ml of 5% sodium
bicarbonate;; b) one time with 30 ml of distilled water;
and c) three times with 30 ml of 5% hydrochloric acid.
These wash solutions were cooled to 4°C to minimize
product hydrolysis. The final organic layer was dried

30 over $MgSO_4$ and the methylene chloride was removed by
roto-evaporation. The crude product was redissolved in
a minimal amount of methylene chloride. Cyclohexane was
added until the mixture was placed in a refrigerator for
one month and additional cyclohexane was added to the

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mixture periodically as the mixtur cl ared, to promote
product crystallization. The white powder which formed
was filtered and dried. A total amount of 0.22 grams,
representing a 6.75% yield of the crystallized product,
was obtained. The product decomposed at between 102°C
and 105°C and was identified by characteristic CH_2 and
SO_2 IR characteristics and $CH_2-(SO_2)_2$, CH_2-O
and $-CH_2-$ proton NMR characteristics.

Example VII
Preparation of Ethylene Methanedisulfonate - Method 2

Tetrahydrofuran, obtained from Burdick and
Jackson Laboratories was freshly distilled from sodium
benzophenone according to standard procedure. A
solution of ethylene glycol obtained from Aldrich
Chemical Co. (1.24 gram) in 200 ml tetrahydrofuran was
added to a 500 ml 3-neck flask filled with a 50 ml
pressure-equalizing dropping funnel connected to a
drying tube, a mechanical stirrer and a low temperature
thermometer. The solution was cooled to -20°C and 4.26
gram (0.02 mole) of methanedisulfonyl chloride in 50 ml
tetrahydrofuran was added from the dropping funnel over
a period of 15 minutes. Collidine, obtained from
Eastman Organic Chemicals (4.85 g, 0.04 mole) in 120 ml
tetrahydrofuran was then added slowly to the flask over
a period of about 1 hour. The reaction mixture was
allowed to warm to 10°C and the collidine hydrochloride
which formed was removed by filtration. The filtrate
was concentrated on the rotary evaporator at 20 mm
pressure. The residue was placed under a high vacuum
(1-2 mm) for about 15 minutes, then 50 ml of cold 5% HCl
was added and the mixture was placed in the refrigerator
overnight. Filtration, followed by vacuum drying,
produced 1.02 g, representing a 25% yield of the

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ethylene glycol ester of methanedisulfonic acid, m.p.
165-169°C. The identity of th product was confirmed by
IR and proton NMR spectra.

Example VIII
5   ### Preparation of Ethylene Methanedisulfonate - Method 3
        In a modification of the method described in
Example VII, glyme was used in place of tetrahydrofuran
and triethylamine, in place of the collidine. The
triethylamine hydrochloride was not filtered and the
10   final reaction solution was evaporated under vacuum, and
the residue was taken up in ice cold water and filtered
to give 4.67 g (57% yield) of ethylene
methanedisulfonate from 0.04 mole of methanedisulfonyl
chloride. This product was further purified by vacuum
15   sublimation at 0.5 to 1.0 mm of Hg in a bath heated to
95°C-102°C. Sublimation removed one of the impurities
noted in the NMR spectrum. The sublimed material was
submitted for biological testing.

Example IX
20   ### Preparation of Trimethylene Methanedisulfonate
        #### Method 2
        The procedure described in Example VIII,
substituting 1,3-propanediol for ethylene glycol, was
followed with glyme as the solvent and triethylamine as
25   the base. The residue, after evaporation of the glyme,
was taken up in methylene chloride and washed
successively with sodium bicarbonate, water, and 5%
hydrochloric acid. After drying the methylene chloride
over anhydrous magnesium sulfate, cyclohexane was added
30   to induce crystallization. From 25.6 g (0.12 mole) of
1,3-propanediol and 24.3 g (0.24 mole) of triethylamine,
2.6 g (10% yield) of trimethylene methanedisulfonate was

obtained. The compound was id ntified by m.p. at
139°C-142°C (dec), and by IR and NMR spectra.

Example X
Preparation of Tetramethylene Methanedisulfonate
5   Method 2
The same procedure as described in Example
VIII, substituting 1,4-butanediol for ethylene glycol,
was used, and from the same molar quantities of reagents
there was obtained a 7% yield of the ester, identified
10 by m.p. 135°C-136°C (dec), and by IR and NMR spectra.

Example XI
Preparation of Pentamethylene 1,1-Ethanedisulfonate
1,5-Pentanediol (4.17 g, 0.04 mole) was
dissolved in 350 ml of glyme in a 1 liter round bottom
15 flask and the solution brought to -20°C.
1,1-ethanedisulfonyl chloride, synthesized in the same
manner as the methanedisulfonyl chloride, (Example I)
was dissolved in 25 ml of glyme and this solution added
dropwise to the solution in the flask. Triethylamine
20 (8.08 g, 0.08 mole) was dissolved in 125 ml of glyme and
this solution was added to the pentanediol/
ethanedisulfonyl chloride solution over a 1 hour
period. After the additions were completed, the mixture
was brought to 25°C over a 45 minute period in a water
25 bath. The mixture was roto-evaporated under reduced
pressure, at a temperature below 35°C. The residue was
washed three times with 20 ml of 5% sodium bicarbonate
the resulting emulsion being separated by
centrifugation. Water was decanted from the final oil
30 wash, and methylene chloride was added. This solvent
apparently dissolved all or most of the impurities,
leaving the product suspended in the solution. The

product was obtained by vacuum filtering the solution
through Whatman #5 qualitative filter papers.
        The dried product weighed 0.22 grams,
representing about a 2% product yield. The product was
5   identified by characteristic IR and proton NMR spectra.
Product decomposition occurred between 141°C and 142°C.
The limited solubility of the compound was confirmed by
dissolving 0.03 grams of the dried product in 1 ml
acetonitrile and in 1 ml of methylene chloride. In each
10 case, evaporation of the supernatant decanted from the
undissolved solid showed that less than 0.01 grams of
the pentamethylene ethanedisulfonate product had
dissolved in each solvent.

                          Example XII
15   Preparation of Tetramethylene 1,1-Ethanedisulfonate
        1,4-Butanediol, obtained from Aldrich Chemical
Co. (3.6 g, 0.04 mole) was dissolved in 75 ml of glyme
in a 1 liter round bottom flask. A solution of
1,1-ethanedisulfonyl chloride (9.1 g, 0.04 mole)
20 dissolved in 25 ml of glyme was added to the flask
through a dropping funnel. The reaction mixture was
kept below -20°C with a dowanol-dry ice bath.
Triethylamine (8.08 g, 0.08 mole) dissolved in 100 ml of
glyme was added to the mixture through a 125 ml dropping
25 funnel over a period of 1 hour. Care was taken to
maintain anhydrous conditions by covering the dropping
funnels with drying tubes containing $CaCl_2$. The
reaction mixture was brought to 25°C in a cold water
bath. The glyme was removed by roto-evaporation, at a
30 temperature below 37°C. The oily yellow residue that
remained after removal of the glyme was washed once with
100 ml of 5% sodium bicarbonate and once with 50 ml of
cold distilled water, the resulting emulsion being

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centrifuged immediately t separate the product, and
aqueous material decanted. The remaining precipitate
was dried under vacuum. The white, powdery solid which
remained weighed 0.90 g, representing a 9.2% yield.
5 Product identification was confirmed by characteristic
IR and proton NMR spectral features. The product
decomposed between 115 and 138°C. The product was
soluble to less than about 0.01 gram in 1 ml of either
methylene chloride or acetonitrile.

10   Example XIII
         Preparation of Trimethylene 1,1-Ethanedisulfonate
             1,3-Propanediol obtained from Aldrich Chemical
    Co. (6.1. g, 0.08 mole) was dissolved in 350 ml of
    distilled glyme in a 3-neck, 1 liter round bottom flask
15 equipped with a stirrer and a thermometer. The solution
    was maintained at -20°C in a dowanol-dry ice bath placed
    underneath the flask. A solution of
    1,1-ethanedisulfonyl chloride (18.2 g, 0.08 moles)
    dissolved in 25 ml of glyme was slowly added through a
20 60 ml dropping funnel. Following this addition, a
    mixture of triethylamine (16.2 g, 0.16 mole) dissolved
    in 125 ml of glyme was added dropwise to the vessel over
    a 1 hour period. Care was taken to avoid contact with
    water by covering the dropping funnels with drying tubes
25 filled with $CaCl_2$. Once all additions were completed,
    the reaction mixture was brought to room temperature and
    stirred for 3 hours.
             The reaction mixture was vacuum filtered to
    remove solid triethylamine hydrochloride. The filtrate
30 was roto-evaporated at a temperature below 37°C to
    remove glyme. The crude product, having a weight of
    7.95 g, was redissolved in a minimal amount of methylene
    chloride, and cyclohexane was added until the mixture

turned cloudy. The initial crystals which formed were
removed from solution by vacuum filtrati n, and
additional cyclohexane was added to produce a second
crop of crystals, which was also removed by filtration.

5 The two crops of crystals were washed with cold
distilled water to remove the surface film of oil. The
final weight of solid obtained represented a 36% product
yield. The sample decomposed between 151°C and 155°C,
when added to an already heated melting-point

10 apparatus. Product identity was confirmed by IR
spectral features related to CH_3CH and SO_2, and by
proton NMR features related to CH, CH_2O, $-CH_2-$ and
$-CH_3$.

Example XIV
15   ## Preparation of Ethylene 1,1-Ethanedisulfonate

The reaction procedure described in Example
XIII for the preparation of trimethylene
1,1-ethanedisulfonate was followed, substituting for the
1,3-propane diol used in Example XIII, ethylene glycol

20 (5.0 gram, 0.008 mole). The reaction mixture was
stirred for 3 hours at room temperature, the solid amine
hydrochloride residue removed, and the filtrate
roto-evaporated to remove glyme. The crude product
obtained was redissolved in a minimum amount of

25 methylene chloride, and subsequent addition of
cyclohexane produced white crystals immediately.
Several crops of crystals totaling 5.84 grams were
obtained after further addition of cyclohexane and
refrigeration. Further recrystallization of the

30 material resulted in a total of 4.37 grams of product,
representing a 25.2% yield. The product melting point
was between 92°C and 93°C. Product identification was
confirmed by IR spectral features relating to CH_3CH

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and SO_2 and proton NMR features relating to CH, CH_2
and CH_3.

Example XV
Preparation of Sodium 1,2-bis (oxysulfonylmethane
sulfonate) Ethane

5   Methanedisulfonyl chloride, 25.0 g (0.117
mole), was placed in a 500 ml round-bottom flask
containing 200 ml of anhydrous ether. To this stirred
solution was slowly added 2.1 g (0.117 mole) water while
10 the solution was cooled in an ice bath. After the
addition of water, the ice bath was removed and the
solution was stirred for four hours. The ether was
removed by rotary evaporation, and 37 g (0.35 mole) of
freshly distilled trimethylsilyl chloride obtained from
15 PCR Research Chemicals, Inc., (Gainesville, FL) was
added slowly, using a bubbler to monitor gas evolution.
After the addition of trimethylsilyl chloride, the
solution was heated to reflux for several hours until
gas evolution ceased. Excess trimethylsilyl chloride
20 was removed by evaporation and the residue was
fractionated, giving 24 g (77%) of trimethylsilyl
chlorosulfonylmethanesulfonate, bp 102°C-104°C at 0.2 mm
pressure, or 110°C-111°C at 0.4 mm pressure. Product
identification was confirmed by titration and by NMR
25 spectra.
        To a solution of 5.52 g (0.0207 mole) of
trimethylsilyl chlorosulfonylmethanesulfonate in 25 ml
of glyme (freshly distilled from sodium and benzophenane
and cooled to -20°C) was added dropwise a solution of
30 0.62 g (0.01 mole) of ethylene glycol and 1.75 g (0.02
mole) of triethylamine in 25 ml of glyme. The solution
was then allowed to warm to room temperature, filtered,
and the glyme removed by evaporation. Two equivalents

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of sodium bicarbonate in water wer added and. after th
gas evolution had ceased, the aqueous solution was
washed with m thyl n chloride and th n vaporated to
leave a white foamy residue containing the sodium salt
5 of 1,2-bis (oxysulfonylmethanesulfonic acid) ethane.

Example XVI
Anti-cancer Activity of Ethylene Methanedisulfonate

Individual strains of mice identified as having
the following types of cancer were employed in the
10 present study: lymphocytic leukemia (PS), lymphoid
leukemia (LE), melanocarcinoma (B1), human xenograft
(MB) and ovarian carcinoma (M5). The left-hand column
of Table I below identifies the 6 test systems which
were studied, including 2 different lymphoid leukemia
15 groups, identified as LE31 and LE37. For each test
system, 6 to 10 animals received a daily dose of
ethylene methanedisulfonate, at the dosage indicated in
the Dose Range column in the table. The injection route
was either intraperitoneal (IP), intracerebral (IC), or
20 subcutaneous (SC), as indicated in the third column from
the left in the table. The dose range was that found to
be most therapeutically effective against the specified
cancer, using the injection route indicated. An equal
number of mice received a daily administration of the
25 drug delivery vehicle only (controls).

The control and drug-treated animals were kept
on this regimen until all the animals had died, except
that in the few cases where the survival time was more
than three times that of the control, the test animals
30 were designated as cured. The T/C ratio, shown at the
right-hand column in Table I, is calculated as the
percentage ratio of the median number of days of
survival of treated animals (T) divided by the median

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number of days of control animals (C). Thus, for the PS
31 test system, the T/C value of 270 indicates that the
median number of days of survival of treated animals was
270% or 2.7 times the median number of days of survival
5 of untreated animals. In the case of the human breast
xenograft test system (MBG5), the T/C ratio provides a
measure of the tumor size of treated animals versus that
of untreated. The value of 7 means that the tumor of
treated animals averaged about 7% of the growth of that
10 of untreated animals.

<u>TABLE I</u>

	Test System	Dose Range	Injection Route	T/C Med.
	3 PS 31	25-50 mg/kg	IP	270
15	3 LE 31	25-50 mg/kg	IP	271
	06 LE 37	6.25-25.0 mg/kg	IC	183
	3 B 131	12.5-25.0 mg/kg	IP	166
	3 MB G5	50-300 mg/kg	SC	(7)
	3 M5 31 (M)	12.5-50 mg/kg	IP	217
20	3 M5 31 (F)	25-50 mg/kg	IP	267

The data in Table I show that ethylene
methanedisulfonate was effective, at various dose
ranges, in treating all five types of cancer studied,
and that the drug delivery could be achieved by a
25 variety of injection routes.

<u>Example XVII</u>
<u>Anti-Leukemic Activity of</u>
<u>Trimethylene Methanedisulfonate</u>

A test system corresponding to PS 31 in Table I
30 was carried out to test the effectiveness of
trimethylene methanedisulfonate against lymphocytic

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leukemia. Th test protocol was identical to that used
in test system PS31 in Example XVI, with the exception
that th ptimal dos range of betw en 6.5 and 12.5 mg
drug per kilogram body weight was used. The T/C ratio,
5 calculated on the basis of the median number of days
survival of treated and untreated animals, was 160,
indicating that the drug is effective in the treatment
of lymphocytic leukemia, but substantially less so than
ethylene methanedisulfonate.

10   Example XVIII
                 Anti-Leukemic Activity of
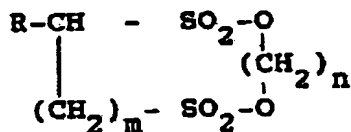   Tetramethylene Methanedisulfonate
       The effectiveness of the tetramethylene
methanedisulfonate compound in treating lymphocytic
15 leukemia in a test system like test system PS 31 of
Example XVI was examined. The test employed the same
general protocol, including the same number of animals
and injection route described in Example XVI for test
system PS 31, except that the optimal drug dosage for
20 the tetramethylene methanedisulfonate compound of 12.5
to 25 mg per kilogram of body weight was administered.
The T/C ratio, calculated on the basis of number of
median number days survival of treated and untreated
animals, was 188, similar to the results obtained with
25 trimethyl methanedisulfonate.
       While the invention has been described with
respect to preferred embodiments and specific examples,
it will be appreciated that various changes and
modifications may be made without departing from the
30 spirit of the invention.

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WHAT IS CLAIMED IS:

1.   A bifunctional alkylating compound comprising a cyclic disulfonic ester of the form

$$R-CH \quad - \quad SO_2-O$$
$$| \qquad\qquad\quad (CH_2)_n$$
$$(CH_2)_m - SO_2-O$$

5   where m=0 or 1, n=1-5, and R=H, CH_3, CH_3CH_2 or Cl.
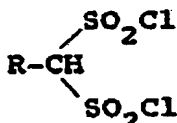
2.   The compound of claim 1, wherein m=0, and R=H, CH_3 or Cl.

3.   The compound of claim 2, wherein m=0 and
10   R=H.

4.   The compound of claim 3, wherein n=2.

5.   The compound of claim 1, wherein n=2.

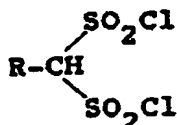6.   The compound of claim 3, formed by the steps of reacting an alkanedisulfonyl chloride of the
15   form

$$R-CH \underset{\diagdown SO_2Cl}{\overset{\diagup SO_2Cl}{}}$$

where R=H or CH_3, with silver carbonate to form the corresponding silver alkanedisulfonate, and allowing the disulfonate to react with a dehaloalkane of the form
20        $X-(CH_2)_n-X$
where X=Br or I, and n=1-5.

7. Th .compound of claim 1, formed by the
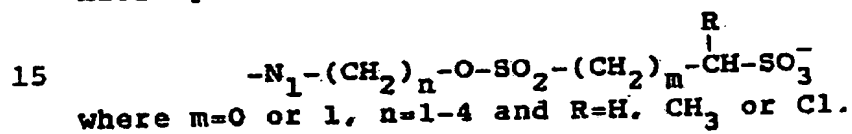steps of allowing an alkane disulfonyl chloride of th
form

$$R-CH \bigg\langle \begin{array}{l} SO_2Cl \\ SO_2Cl \end{array}$$

where R=H or CH_3, to react with an alkane diol of the
5 form

$$OH-(HC_2)_n-OH$$

where n=2-5, in the presence of tetrahydrofuran or glyme
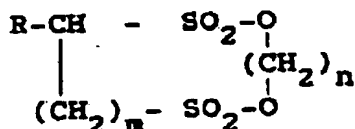and an aliphatic or aromatic tertiary amine.

8. The compound of claim 7, wherein the
10 tertiary amine is added dropwise to the other reactants
at a reaction temperature below about -20°C.

9. A bifunctional alkylating compound capable
of reacting with a first nucleophile $-N_1$ to produce a
nucleophile-reactive intermediate of the form

15
$$-N_1-(CH_2)_n-O-SO_2-(CH_2)_m-\overset{\overset{\textstyle R}{|}}{CH}-SO_3^-$$

where m=0 or 1, n=1-4 and R=H, CH_3 or Cl.

10. The compound of claim 9, wherein n=2 and
R=H.

11. The compound of claim 9, which includes a
20 cyclic disulfonic ester of the form

$$\begin{array}{ccc} R-CH & - & SO_2-O \\ | & & (CH_2)_n \\ (CH_2)_m- & SO_2-O \end{array}$$

where n=1-5.

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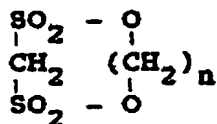12. The compound of claim 9, which includes a
linear disulfonic acid of the form

$$^-SO_3-\overset{\overset{\displaystyle R}{|}}{CH}-SO_2-O-(CH_2)_n-CH_2-O-SO_2-\overset{\overset{\displaystyle R}{/}}{CH}-SO_3^-$$

where n=1-4 and R=H, Ch$_3$ or Cl.

13. An anti-cancer compound comprising a
5 cyclic disulfonic ester of the form

$$\begin{array}{ll} SO_2 & - \ O \\ | & \quad | \\ CH_2 & (CH_2)_n \\ | & \quad | \\ SO_2 & - \ O \end{array}$$
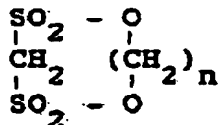
where n=2-4.

14. The compound of claim 13, wherein n=2.

15. The compound of claim 14, for use in
10 treating mammalian lymphocytic leukemia, lymphoid
leukemia, melanocarcinoma, human breast xenograft and
ovarian carcinoma.

16. A method of treating a mammalian cancer
comprising administering a therapeutically effective
15 amount of a cyclic disulfonic ester compound of the form

$$\begin{array}{ll} SO_2 & - \ O \\ | & \quad | \\ CH_2 & (CH_2)_n \\ | & \quad | \\ SO_2 & - \ O \end{array}$$

where n=2-4.

17. The method of claim 16, wherein n=2.

18. The method of claim 17, wherein the cancer
20 includes lymphocytic leukemia or lymphoid leukemia, and
said administering includes administering the compound

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intraperiton ally, at a dose of betwe n about 25 and 50
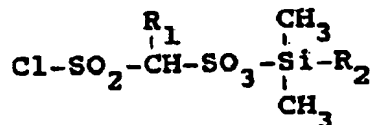milligrams per kilogram of body w ight.

19.   The method of claim 17, wherein the cancer
includes a lymphoid leukemia, and said administering
5 includes administering the compound intracranially at a
dose of between about 6.25 and 25 milligrams per
kilogram of body weight.

20.   The method of claim 17, wherein the tumor
includes a melanocarcinoma, and said administering
10 includes administering the compound intraperitoneally at
a dose of between about 12.5 and 25 milligrams per
kilogram of body weight.

21.   The method of claim 17, wherein the cancer
includes a human breast xenograft and said administering
15 includes administering the compound subcutaneously at a
dose of between about 50 and 300 milligrams per kilogram
of body weight.

22.   The method of claim 19, wherein the cancer
includes an ovarian carcinoma, and said administering
20 includes administering the compound intraperitoneally at
a dose of between about 15 and 50 milligrams per
kilogram of body weight.

23.   A compound of the form

$$Cl-SO_2-\overset{\displaystyle R_1}{\underset{}{\overset{|}{C}H}}-SO_3-\overset{\displaystyle CH_3}{\underset{\displaystyle CH_3}{\overset{|}{\underset{|}{Si}}}}-R_2$$

25 where R_1=H, CH_3 or Cl, and R_2=CH_3 or $C(CH_3)_3$.

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [1]

According to International Patent Classification (IPC) or to both National Classification and IPC

C07C 143/68, 143/70; A61K 31/385, 31/39, 31/255, C07D 327/00; C07F 7/08

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U. S.	260/456 A, 456 R 556/428 424/276, 277, 303 549/11, 19

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category [*]	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X	AT, B, 210/874 Published 25 August 1960	1-15
Y	AT, B, 210/874 Published 25 August 1960	1- 22
Y	GB, A, 700,677 Published 09 December 1953 Timmis	15-22
Y	DE, B, 1,124,032 Published 22 February 1962	9, 12
Y,E	US, A, 4,483,799 Published 20 November 1984, Kampfer et al.	1-15
A,P	US, A, 4,483,799 Published 20 November 1984 Kampfer et al	1-22
A	DE, B, 1,277,247 Published 12 September 1968	1-15
A	N, Chemical and Pharmaceutical Bulletin, issued 01 November 1964, Hayashi et al, Studies on Anti Tumor Substances, Synthesis of Bis-(Methane Sulphonylthio) Alkanes pp 1271-72	1-22

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
10 April 1985	24 APR 1985
International Searching Authority [1] ISA/US	Signature of Authorized Officer [10]